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Lipids

The absorption of fat

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Steatorrhoea may be produced by the interference of a variety of processes which are involved in fat absorption (Senior, 1964; Dawson, 1967; Johnston, 1968). The main dietary lipid is triglyceride and the normal naturally occurring triglyceride tends to have an unsaturated fatty acid in the beta position. The function of the luminal changes in fat is to convert a water-insoluble substance which aggregates in large particles, eg, oil emulsion if predominantly unsaturated fats are fed but debris if the solid tristearin is used, into so finely dispersed a form that it can penetrate between the microvilli and then pass through the microvillous membrane. This is largely brought about by the digestion of the triglyceride and solubilization of these digestion products in an aqueous bile salt solution.

Digestion

This is brought about by the action of pancreatic lipase which is a specific esterase which only acts on an oil-water interface. This was beautifully shown by Desnuelle (1961) and his colleagues using the short-chain triglyceride triacetin and purified rat pancreatic lipase. Hydrolysis was estimated in solutions of increasing concentration. Negligible hydrolysis occurred until the solution became saturated and an emulsion formed (Fig. 1). It has thus been found that in kinetic analyses of lipase action one uses the area of an emulsion rather than the concentration of substrate in, for example, performing a Lineweaver-Burk plot. The esterase, which also occurs in pancreatic juice and acts on water-soluble esters, is a separate enzyme. The occurrence of these two enzymes has in the past caused confusion, for often water-soluble substrates were used to estimate 'lipase'. Lipase attacks the two primary bonds and both these reactions are reversible. Thus, if triglyceride and labelled acid are fed to a person and samples of intestinal contents retrieved, labelled acid will be found in the one and three position of the triglyceride (Ahrens and Borgström, 1956). Complete hydrolysis can take place but this is thought to be after isomerization of the fatty acid in the beta to the alpha position; this reaction is irreversible.

The Role of Bile Salts

Until recently it was assumed that bile salts mainly acted by potentiating the action of lipase either as emulsifying agents or in removing hydrolytic products from the oil-water interface of the emulsion. Such effects are not of great physiological importance for, in the absence of bile, hydrolysis is not rate limiting (Annegars, 1954). In fact, the main function of bile salts seems to be to solubilize the luminal fat—a function suggested by Verzar many years ago (Verzar and McDougall, 1936). Our knowledge of the role of bile salts and the importance of their detergent properties in facilitating fat absorption stems largely from the work of Borgström and

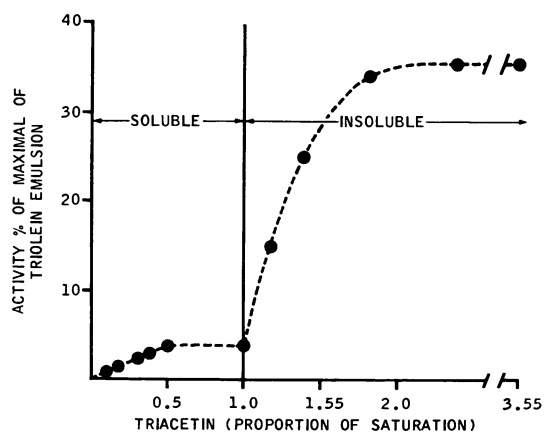
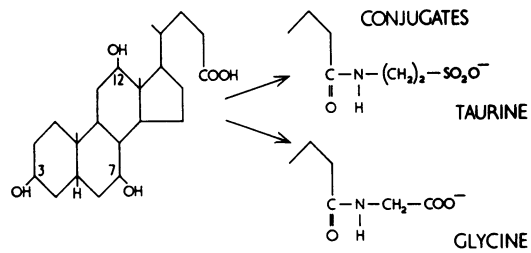


Fig. 1 *Action of pancreatic lipase. Note the rapid increase in hydrolysis when an emulsion forms. (After Desnuelle, 1961.)*

Hofmann (Hofmann and Borgström, 1962; Borgström, 1967; Hofmann and Small, 1967). Bile acids found in man are shown in Figure 2. The so-called primary bile acids are cholic acid, a trihydroxy acid, and the 3,7 dihydroxy acid, chenodesoxycholic acid; the 7 dehydroxylation of cholic acid by bacteria occurs with the formation of desoxycholic acid (3, 12 dihydroxy acid) in the intestinal tract during its enterohepatic recirculation. Under normal physiological conditions the bile acids are present mainly in the form of their conjugates of either glycine or taurine, and, as can be seen from Fig. 2, this conjugation affects the ionic strength of the compound, thus the free acids have a *p*K of approximately 6, the glycine conjugates of 4, and the taurine conjugates of 2. Thus intestinal contents which usually have a *p*H greater than 5 always have the conjugated salts predominantly in the ionized form. From the chemical structure it will be seen that these compounds are detergents or ampipaths. The sterol nucleus is lipid soluble and the hydroxyl groups and ionized conjugate of glycine or taurine are water soluble. When a molecular model is constructed the water-soluble portions are all on one side and the lipid-soluble on the other. Such compounds or ampipaths have two important properties. (1) They are emulsifying agents, the sterol dissolves on the outside of an oil droplet and the water-soluble particles dissolve in the water, the charges between them stabilizing the surface of the emulsion. (2) With increasing concentration, instead of coming out of solution, they aggregate together to form micelles, the lipid-soluble sterol backbone facing inwards and the water-soluble outwards into the water. The concentration at which this occurs is known as the critical micellar concentration. This varies for each bile salt but for the dihydroxy conjugates it is approximately 1·5-2 mM and for the trihydroxy conjugates 4-5 mM. The critical micellar concentration of the mixture of bile salts which occurs in intestinal contents is about 3 mM. Once bile salts have aggregated into micelles they can solubilize other lipids in their interstices.

With regard to dietary fat, triglyceride is virtually insoluble in micelles but the digestion products, monoglyceride and fatty acid, are readily incorporated. It is thought that the polar glycerol backbone points to the outside and the hydrocarbon to the inside of the micelle. Some very insoluble fatty acids, such as stearic acid and its monoglyceride, and lipids, such as cholesterol and fat-soluble vitamins, are virtually insoluble in pure bile salt micelles but are readily soluble in mixed micelles which have been expanded by the incorporation of monoglycerides and fatty acids of unsaturated fatty acids or saturated fatty acids C16 and below. It should be pointed out that the monoglyceride with the greatest solubility



	Hydroxyl group	Bile acid	pK Free	pK Conjugates Glycine	pK Conjugates Taurine
TRIHYDROXY	3α 7α 12α	Cholic	6	4	2
DIHYDROXY	3α 7α	Chenodeoxycholic	6	4	2
	3α 12α	Deoxycholic	6	4	2

Fig. 2 *The main bile salts found in human bile.*

in micelles is an unsaturated monoglyceride, which is appropriate when one remembers that triglycerides usually have such fatty acids in the beta position.

The physiological importance of mixed (or expanded) micelles in solubilizing lipids virtually insoluble in pure bile salt solutions is shown in some experiments with stearic acid. It is known that this fat fed either as the free acid or triglyceride is poorly absorbed but when fed with unsaturated acids the absorption is increased (Renner and Hill, 1961). At one time it was thought that the stearic acid dissolved in the oleic acid. In recent experiments it has been confirmed that the solubilization of stearic acid in bile salt micelles is increased by the presence of mono-olein, while the stimulation of the stearic acid absorption in rats fed triolein does not occur in the bile fistula rat (Fig. 3) (Hamilton, Webb, and Dawson, 1969). Furthermore, Simmonds, Hofmann, and Theodor (1967) showed that when the upper intestine of man was perfused by a mixed micellar solution of conjugated bile salts, glyceride hydrolysis products, and cholesterol the poorly absorbed cholesterol came out of solution once the monoglyceride and fatty acid had been absorbed in the upper intestine even though the bile salt concentration had remained virtually unaltered.

One may look on intestinal contents as containing three major phases in terms of bulk, that is, the oil phase, the micellar phase, and debris formed from unabsorbed solid food and shed cells. These are in continuous equilibrium. Molecules passing from the oil or debris to micelle must presumably pass through a small but active rapidly turning over molecular or truly aqueous phase. Using either high speed centri-

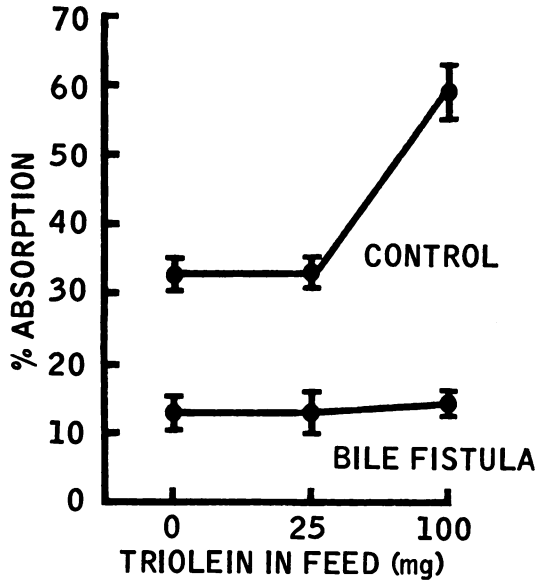


Fig. 3 *Absorption of 25 mg tristeamin-1-^{14}C in the presence of triolein* (*Hamilton* et al, *1969*).

fugation techniques in which micelles remain in the infranatant clear fluid and the oil floats to the top of millepore filters one can show that there is equilibration between oil and micellar phases and, as might be expected, an increase in bile salt concentration or an increase in *p*H (with a tendency to greater ionization of any fatty acid) will alter the partition of fatty acids in favour of the micellar aggregates (Borgström, 1967). Various attempts have been made to assess the size of pure and mixed micelles by centrifugation (Small, 1968), the use of gel filtration (Feldmann and Borgström, 1966), and measurement of the rate of diffusion (Woodford, 1969); the diameter of pure and mixed micelles probably ranges from 4 to 40 Å. These can easily pass between microvilli which are approximately 200 Å apart. Thus the whole of the microvillous surface should be accessible for the absorptive process, although what effect the surface coat or fuzz has on the movement of micelles and any other particle between microvilli is unclear.

The mechanism by which fat is transferred from the micellar to microvillous membrane is still open to debate (Webb, Hamilton, and Dawson, 1969). Either there is equilibrium between the micellar phase and the lipid membrane via the molecular phase, or the whole micelle has to come into contact with the membrane where it would either be immediately disrupted. In this case the bile salt and some of the less well absorbed lipid pass back into the lumen while the fatty acid and monoglyceride stays attached to and then passes through the membrane, or the whole micelle may even pass into the cell. The latter is probably unlikely as it is known that conjugated bile salts are very poorly absorbed by the jejunal mucosa. However, they may well get across the jejunal mucosal membrane even if they cannot pass the serosal side of the cell. Certainly there is evidence of bile salts within the jejunal mucosa.

Although the importance of bile salts has been stressed in the absorption of fat it must be pointed out they are not obligatory for the absorption of the hydrolytic products of triglyceride. In the experiments of Annegars (1954), up to 50% of fat was absorbed in the absence of bile and this has been found in patients with obstructive jaundice and also in many other animal experiments (Gallagher, Webb, and Dawson, 1965). However, the absorption of certain lipids, such as fat-soluble vitamins and cholesterol, is far more dependent on the presence of bile (see Thompson, this symposium). The mechanism whereby the hydrolytic products of triglycerides are dispersed in the absence of bile is still open to debate but protein degradation products may well be important.

The Enterohepatic Circulation of Bile Salts (Fig. 4)

It is usually considered that bile salts are the main products of cholesterol oxidation in the liver. About 0·8 g is formed in a day and this is immediately conjugated with either taurine or glycine. The glycine to taurine ratio is 3:1. The total bile acid pool

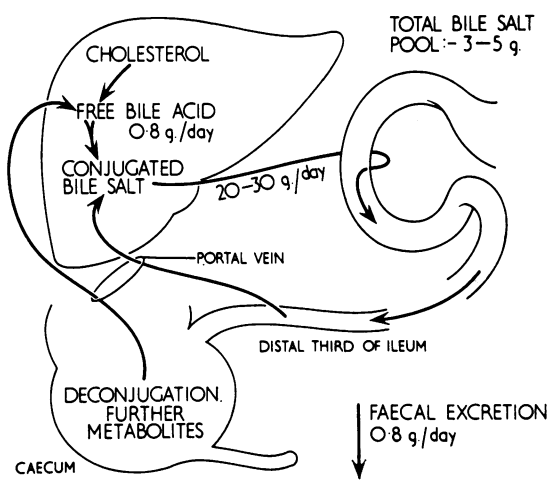


Fig. 4 *Enterohepatic circulation of bile salts* (*after Bergstrom and Danielsson, 1968*).

is 1-3 g and it has been calculated that 20-30 g is excreted in the bile each day; the total pool recirculates 5-10 times. There is negligible absorption of the taurine conjugates in the jejunum, and only up to 20% of the glycine conjugates is absorbed there so that a high bile salt concentration usually well above the critical micellar concentration is maintained in that portion of the small bowel from where fat is absorbed. A specialized mechanism for the absorption of bile salts is present in the ileum (Lack and Weiner, 1963) which, to a large extent, prevents the escape of bile salts into the colon. Bacteria are present both in the terminal ileum and the colon. Some species of bacteria deconjugate and also dehydroxylate the bile acids. Such metabolic products are partially absorbed by the colon and approximately 0·8 g of bile salt is lost in the stool per day. Thus even in normal subjects 15-40% of intravenous taurocholate may have been shown to be deconjugated and partially dehydroxylated and reconjugated with glycine within 24 hours (Garbutt, Wilkins, Lack, and Tyor, 1970).

Cellular Phase

Once the monoglyceride and fatty acid has entered the mucosal cell it is mainly reconverted into triglyceride (Dawson, 1967; Johnston, 1968). This takes place via two pathways. One of these, the so-called alpha glycerophosphate pathway, is present in most cells capable of synthesizing triglyceride. Alpha glycerophosphate is acylated by fatty acids activated with CoA (Brindley and Hubscher, 1966) to form phosphatidic acid which is then dephosphorylated to form diglyceride which is further acylated to form triglyceride. But in addition there is a specialized mechanism whereby monoglyceride can be directly acylated with acyl-CoA derivatives thus preventing the need to break down the remaining original glyceride bonds and expend energy on resynthesizing them (Clark and Hubscher, 1961; Senior and Isselbacher, 1962). It is thought that about 60% of the triglyceride backbone is preserved by this monoglyceride acylase pathway. It seems likely that the relative utilization of the two paths is dictated by the availability of the various substrates in the mucosal cell. Thus, when there is excess fatty acid the alpha glycerophosphate pathway is probably used, when there is an equal amount of monoglyceride and fatty acids the monoglyceride pathway is used, and when there is an excess of monoglyceride this is hydrolysed by specific monoglyceride lipase (Senior and Isselbacher, 1963). It seems probable that the extent of total glyceride hydrolysis is a measure of these intracellular processes rather than luminal lipase activity.

Once formed, the triglyceride is mainly incorporated into chylomicrons which have a partial coat of lipoprotein together with cholesterol ester and phospholipid but it is now realized that it may also be incorporated into very low density lipoprotein (Ockner, Hughes, and Isselbacher, 1969) and that both moieties may be used to transport the fat from the cell via the lymph into the systemic circulation. It seems probable that even under normal conditions a small amount of lipid is transported by the portal veins, most likely as the free fatty acid. After feeding radioactive lipid a small proportion of lymph radioactivity, up to 3%, is found as free fatty acid. This must mean that free fatty acid can pass through the mucosal cell and this being so, if bound to albumin, then it is as likely to enter the portal blood as the lymph (Dawson, Gallagher, Saunders, and Webb, 1964).

The Causes of Steatorrhoea

These are listed in Table I. They may be divided into disturbances of the luminal and mucosal phase of absorption.

Phase of Absorption	*Cause of Steatorrhoea*
Luminal	
	Digestion
	Pancreatic insufficiency
	Inactivation of lipase by low *p*H
	Bile-salt deficiency
	1 Impaired entry to lumen due to:
	Hepatocellular disease
	Malnutrition
	Obstructive jaundice
	2 Loss from lumen:
	Stagnant loop syndrome
	Ileal disease or resection
	Cholestyramine
	Miscellaneous
	Neomycin
Mucosal	
	Loss absorbing site
	Loss bile salt absorbing site
	a β lipoproteinaemia
Lymphatic obstruction	
Unclassified	*eg*, phenolphthalein, PAS, addiction to nuts, thyrotoxicosis

Table I *Causes of steatorrhoea*

THE LUMINAL PHASE

Impaired lipolysis

This is usually due to pancreatic insufficiency secondary to chronic pancreatitis or following resection of a pancreatic carcinoma. Occasionally it is due to the Zollinger-Ellison syndrome, when the massive gastric hypersecretion causes a very low *p*H in the duodenum and jejunum with irreversible inactivation of the *p*H-sensitive lipase (Go, Poley, Hofmann, and Summerskill, 1970). The differentia-

tion of pancreatic insufficiency from other causes of steatorrhoea is most simply demonstrated by the Lundh test (1962) in which a mixture of milk powder, corn oil, and glucose is fed and samples of intestinal contents are analysed for trypsin. There is a sharp cut-off point between patients with pancreatic and other forms of steatorrhoea (Fig. 5), although it should be pointed out that patients with pancreatitis and without any evidence of exocrine insufficiency may not have greatly diminished trypsin in the lumen. Trypsin is more frequently measured than lipase for technical reasons. If the samples of intestinal contents are collected in ice and glycerides and fatty acids separated by thin layer chromatography samples from normal subjects contain predominantly free fatty acid; but samples from those with pancreatic insufficiency will have a larger triglyceride spot in the chromatogram. Patients with pancreatic insufficiency will have a great proportion of oil phase in the intestinal lumen.

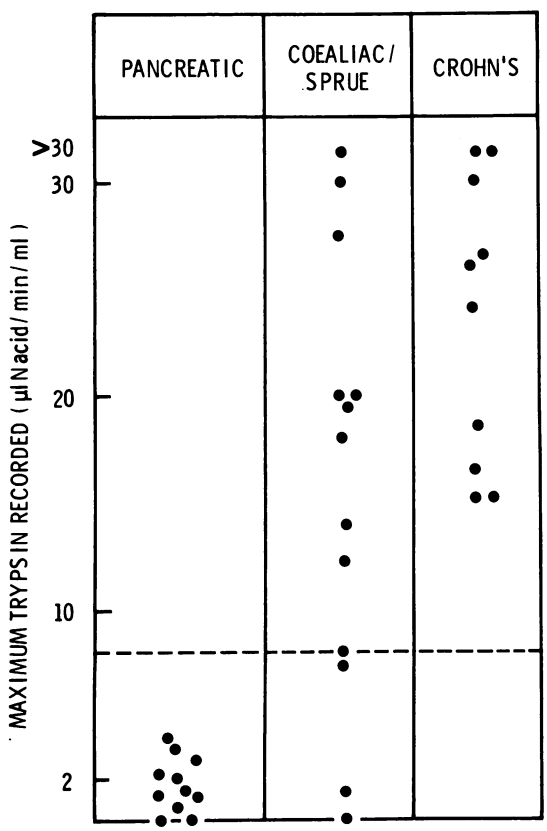


Fig. 5 *Pancreatic function in steatorrhoea (Lundh test). Two patients with coeliac disease had coincidental pancreatic insufficiency. (G. E. G. Sladen, J. P. W. Webb, and A. M. Dawson, unpublished observations.)*

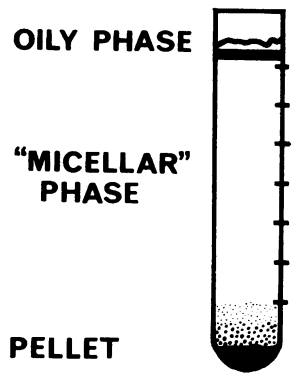
Therefore fat-soluble vitamins will tend to partition into the oil phase rather than the micellar phase, especially as the micelles will tend to be those of pure bile salts rather than be expanded by pancreatic lipolytic products. This may account for the occasional presence of osteomalacia and other fat-soluble vitamin deficiency states in such subjects.

Luminal bile salt deficiency

This may be due to the impaired entry of bile salts into the duodenum in the presence of liver disease and possibly in malnutrition when bile salt synthesis is depressed or there is obstruction to the hepatic ducts. On the other hand, in patients with ileal resection there is interruption of the normal enterohepatic circulation of bile salts giving rise to excessive loss from the small bowel. This is degraded by bacteria in the colon and only partly reabsorbed. Thus it can be shown using tracer techniques that the half-life of the bile salts may be grossly reduced (Austad, Lack, and Tyor, 1967), that if labelled taurocholate is used an abnormally high proportion of label appears in metabolized bile salt, ie, dihydroxy and glycine conjugates (Garbutt *et al*, 1970), and when loss outstrips the liver's synthetic capacity the total bile salt pool is depleted so that eventually very low concentrations occur in the jejunal lumen. The unabsorbed bile salts inhibit salt and water by the colon (Hofmann, 1967; Mehkjian and Phillips, 1970) so that the diarrhoea caused by steatorrhoea may be aggravated or diarrhoea can occur even in the absence of an abnormal faecal fat; this may be ameliorated by giving the bile salt-binding resin cholestyramine (Hofmann and Poley, 1969).

In the blind loop syndrome the normally relatively sterile small bowel is infested with colonic bacteria. Some strains of species have the ability to split and dehydroxylate bile salts giving rise to a high concentration of unconjugated bile acids in the jejunal lumen. These acids are absorbed from the jejunum by non-ionic diffusion so that the total luminal bile salt concentration falls below the critical micellar concentration and steatorrhoea may follow (Tabaqchali and Booth, 1970). At least two pharmacological agents interfere with micelle formation. The bile salt-binding resin, cholestyramine, when given orally breaks the enterohepatic circulation of bile salts and when given in large enough doses to relieve the pruritus of obstructive jaundice may so reduce the bile salt concentration as to cause steatorrhoea (Hofmann and Poley, 1969). The polybasic antibiotic neomycin is poorly absorbed and when given in large doses, such as are often used in hepatic coma, can cause steatorrhoea. This is mainly due to disruption of micelles by binding the fatty acid moiety (Thompson, MacMahon, and Claes, 1970).

It has been possible to confirm these theoretically predictable disturbances of luminal bile salt metabolism and micelle formation by analysis of intestinal contents. This depends on good methods for separating and measuring the total concentration of bile salts and also separating the micellar phase from the oil and debris of intestinal contents. The former has been achieved by thin layer chromatography and the use of 3 hydroxy steroid dehydrogenase (Turnberg and Mote, 1969). The latter is a more formidable problem. Intestinal contents are usually heated to 70°C to inactivate lipase and then the oil is separated from the micellar phase by high speed centrifugation (Hofmann and Borgström, 1964). One should be



BILE SALTS (mM)	FFA (mM)	TOTAL P (mM)
1.03	3.51	0.20
1.95	4.48	0.27
2.53	5.36	0.31
6.36	11.50	2.01
14.94	72.15	8.26
18.06	82.68	10.96

Fig. 6 *Gradient of bile salt and lipid concentration after centrifugation of intestinal micellar phase (after Porter and Saunders, 1971).*

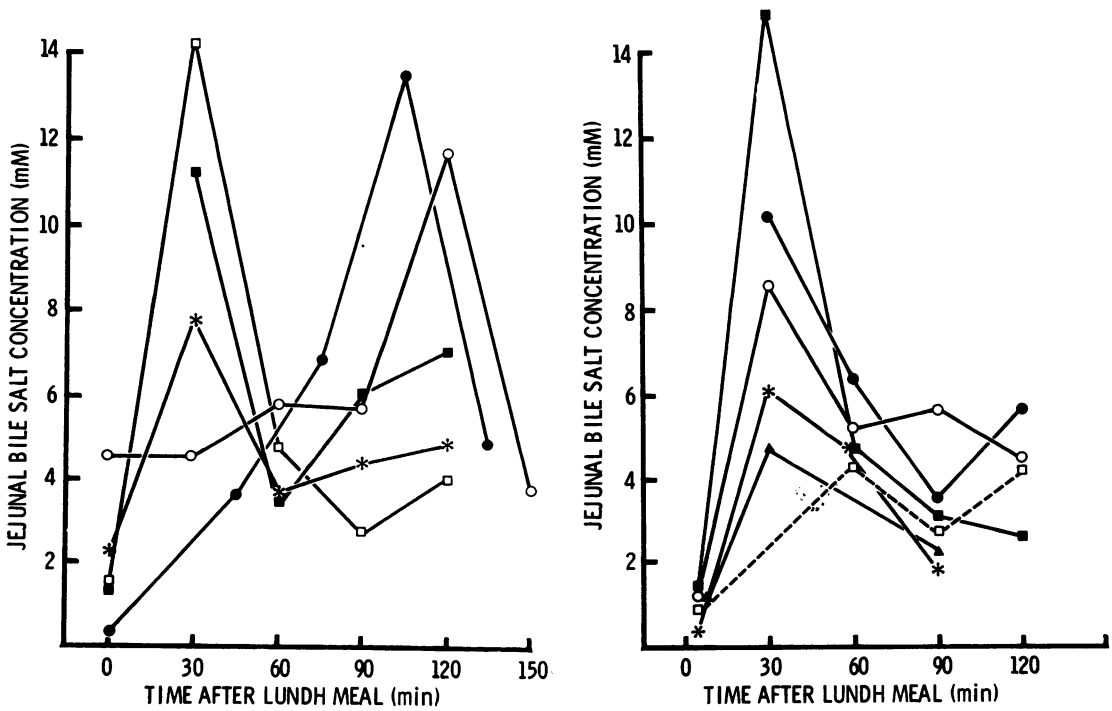


Fig. 7 *Variation of jejunal bile salt concentration at various times after feeding a Lundh meal:* left, *in five normal subjects;* right, *repeated observations on one normal subject (J. D. Hamilton, J. P. W. Webb, and A. M. Dawson, unpublished observations.)*

able to predict roughly the micellar concentration of lipid knowing the bile salt concentration, *p*H, and type of lipid involved in the light of experiments *in vitro* (Borgström, 1967; Hofmann and Small, 1967). Although good predictions have been found (van Deest, Fordtran, Morawiski, and Wilson, 1968), often they are rough (Bradley, Murphy, Bouchier, and Sherlock, 1970) or virtually non-existent (Krone, Theodor, Sleisinger, and Jeffries, 1968). Recently Porter and Saunders (1971) have published an extremely important methodological paper which may partly explain these discrepancies. They showed that when high speed centrifugation is used there is a tremendous concentration gradient of lipid and bile salt from top to bottom of the tube. For example, there may be a sevenfold variation in cholesterol concentration and comparable differences in bile salt and fatty acid composition (Fig. 6). Furthermore, the usual method of inactivating the explosive lipolysis induced by the high concentrations of luminal lipase, that is heating to 70°C, was shown to increase lipolysis further. When a meal containing protein was fed it was impossible to separate off the micellar phase with millipore filters, as the protein clogged the filters. They have now developed a method in which, after feeding a protein-poor meal, small-intestine contents can be rapidly processed through a series of millipore filters so that the micellar phase is homogeneous and lipolysis minimal. Luminal bile salt concentrations also vary widely at different times after a meal. This may be partly due to emptying of the gallbladder (Fig. 7a). Unfortunately, the pattern is not even reproducible in a single subject (Fig. 7b), so that great caution must be exercised in interpreting the glut of data which is appearing on this subject, especially when the effects of treatment, eg, of the blind-loop syndrome, are being claimed. Incorporation of a marker to the lipid meal is fraught with theoretical difficulties, for any marker which is not lipid soluble empties from the stomach at a different rate as it separates from oil and debris (Wiggins and Dawson, 1961). If it is lipid soluble it does not enter the micellar phase (Morgan and Hoffman, 1970) so that adequate mixing of contents and marker is again impossible.

The Mucosal Phase

The usual cause of interference with the mucosal phase is when it is resected, inflamed, or bypassed either at the site of fat absorption or where bile salt reabsorption takes place. There is only one specific biochemical defect—a β lipoproteinaemia in which exit of resynthesized triglyceride from the mucosal cell is impaired by lack of synthesis of the lipoprotein of very low density lipoprotein and chylomicrons (Isselbacher, Scheig, Plotkin, and Caulfield, 1964). Exit of triglyceride from the mucosa cell may also be impaired in diseases of the intestinal lymphatics.

Miscellaneous

Steatorrhoea occasionally occurs in purgative addicts, for phenolphthalein has been shown to produce steatorrhoea on occasion (French, Gaddie, and Smith, 1956). Recently a patient who was addicted to eating nuts also presented with unexplained steatorrhoea (Bamforth, Murray, and Roberts, 1967). The mechanism whereby para-aminosalicylic acid interferes with fat absorption is unknown (Coltart, 1969).

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